Original Article

Expression of CD3 and CD20 in Antistreptolysin-O Titer Seropositive and Seronegative Children with Chronic Tonsillitis

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Abstract

Background: Chronic tonsillitis (CT) is a common inflammatory illness in children, and serum antistreptolysin O titer (ASOT) is a common investigation performed for these cases and considered a perfect sign for tonsillectomy. **Objective:** To evaluate the expression of tonsillar T-and B-lymphocytes markers in relation to seropositive or seronegative ASOT in cases of CT. **Materials and Methods:** Thirty children (15 males and 15 females) aged 6–10 years were divided equally into two groups: Group A seropositive ASOT (≥400 IU) and Group B seronegative ASOT (<400 IU). Both performed bilateral tonsillectomy. Specimens from the removed tonsils were taken and prepared for light microscopic examination and immunohistochemical evaluation of CD20 and CD3 expression. **Results:** Seropositive ASOT group showed significant histopathologic changes in the form of hyperplasia of the stratified squamous nonkeratinized epithelium, Urgas's abscess, and severe lymphocytic infiltration. Immunohistochemical results of seropositive ASOT group showed marked expression of CD3 and CD20, while seronegative ASOT group showed mild expression of CD3 and CD20. **Conclusion:** Seropositive ASOT CT, in addition to histopathological changes, is associated with significant increase in both B-lymphocytes (CD20 expression) and T-lymphocytes (CD3 expression) markers.

Keywords: Antistreptolysin-O titer, CD20, CD3, chronic tonsillitis, immunohistochemistry, tonsillectomy

Introduction

Chronic tonsillitis (CT) is a common chronic inflammatory disease of the palatine tonsils that requiring surgical removal of the diseased tonsils.[1] It can bring about multiple complications in children both nearby provincial spreads such as acute rhinitis, sinusitis, and descending respiratory infections or at the distance such as glomerulonephritis, joint rheumatism, rheumatic fever, endocarditis, and appendicitis.[2]

Palatine tonsils are placed in the oropharyngeal inlet passageway for invulnerable protection against took in and breathed pathogens. Histologically, the tonsil made of mass of lymphoid follicles supported on a connective tissue framework; the tonsillar crypts penetrate from the surface, down to the center of the tonsil follicle. The luminal surfaces of the tonsils are covered with nonkeratinizing stratified squamous epithelium, which is the same tissue of the surrounding oropharynx. Protection of this zone relies upon both inborn and acquired immune responses. As indicated by their lymphoid nature, tonsils are the introductory destinations for both humoral (B-lymphocytes) and cell-mediated (T-lymphocytes) immune reactions. T-lymphocytes, specifically, are available in plenty in palatine tonsils and are to a great extent situated in the extrafollicular zones. Tonsils have been portrayed as destinations of acceptance of oral invulnerable resistance. In spite of its visionary significance, there are high quantities of tonsillectomy medical measures done to avoid complications result from tonsillitis.[3]

Histopathologically, in CT, the involved tonsils became congested, hypertrophied, or covered with white-faint bogus membranes. The tonsillar lymphoid follicles showed up as hyperplastic and hypertrophic with excessive development of the germinal clear center.[4]

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The most ordinarily used investigation for CT is serological detection of antistreptolysin-O titer (ASOT), a toxic enzyme produced by Group A beta hemolytic streptococcal (GABHS). This test is especially used in school-age children who are progressively susceptible to frequent streptococci infections because of its accessibility, less price, and reasonable sensitivity.[5] Hembrom *et al*. thought about the affectability of high ASOT (seropositive) with throat swab and tonsillar core culture and found that ASOT sensitivity is 100%. They considered it as a valid and dependable tool for the diagnosis of recurrent tonsillitis and a perfect indication for tonsillectomy.[6] Seropositive ASOT was found to be associated with increased number of circulating T-lymphocytes, which can lead to serious complications. However, many cases with seronegative ASOT were forced to undergo tonsillectomy.[7] Up to our awareness, barely any studies have been made to correlate between tonsillar histopathological changes and CD3 and CD20 markers' expression with changes in ASOT levels.

The aim of our study was to evaluate T- and B-lymphocytes (tonsillar lymphoid nodules) CD3 and CD20 markers' expression in relation to seropositive or seronegative ASOT.

Materials and Methods

This study was conducted at Ear, Nose and Throat (ENT) and Histology Departments, Faculty of Medicine, Jazan University, KSA, and Faculty of Medicine, Suez Canal University, Egypt, respectively.

Ethical considerations

Written informed consent was acquired from first-degree relatives of the children. The local ethics committee in both faculties approved the study.

Selection of patients

Thirty children (6–10 years), including 15 males and 15 females, who made tonsillectomy in the period from October 2017 to April 2018, were included in this study. The indications for tonsillectomy are as follows:

(1) Recurrent attacks of acute tonsillitis: In the form of at least five attacks per year for the last two successive years. (2) Obstructive sleep apnea: when there were huge tonsils associated with mouth breathing, snoring, and history of recurrent apnea during sleep. (3) Peritonsillar abscess (quinsy): After second quinsy, tonsillectomy was recommended. (4) Unilateral tonsillar enlargement.[1] Medical history and ENT examination of the patients were done 1 day before surgery and routine investigations included hemoglobin level, bleeding and clotting time, partial thromboplastin time, and ASOT.

Exclusion criteria were general contraindications for tonsillectomy such as bleeding tendency or acute infection.

Clinical grade classification

Clinical tonsillar grading was done according to Dell'Aringa *et al*., grading classification which depends on the percentage of air obstruction caused by enlarged tonsils as follows: Grade 0 (intravelic tonsils), Grade 1+ (25% air obstruction due to protruding tonsils out of tonsillar fossa), Grade 2+ (25%–50% air obstruction), Grade 3+ (50%–75% air obstruction), and Grade 4+ (75% air obstruction).[8]

Serological test

Children were set into two equal groups according to ASOT results: Group A seropositive ASOT (≥400 IU) and Group B seronegative ASOT (<400 IU). ASO latex agglutination test was done for the measurement of antibodies to antistreptolysin-O in human serum. The presence of an ASOT of 400 IU/mL or higher in the serum gives a visible agglutination of the latex particles.[9]

Surgical operation

For all patients, bilateral tonsillectomy was performed under general anesthesia. Antibiotic administration was achieved (ceftriaxone at a dose of 50 mg/kg) intraoperatively and was continued for 10 days postoperatively (amoxicillin and clavulanate 90 mg/kg).

Histopathological studies

The removed tonsils were identified as right and left and fixed for 24 h in 10% neutral-buffered formalin. Specimens from each tonsil including surface epithelium were taken (5 pieces/each tonsil). Sections from the right tonsil paraffin blocks of 5 μm thickness were prepared and stained with Hematoxylin and Eosin (H and E) and Masson's trichrome stain. Samples from the left tonsil were processed for immunohistochemical staining of CD20 (Monoclonal Mouse Anti-Human CD20, Dako, USA) and CD3 (Polyclonal Rabbit Anti-Human CD3, Dako, USA) in order to reveal B-lymphocytes and T-lymphocytes. Sections were deparaffinized, rehydrated, and incubated in 0.3% H_2O_2 for 1 h, for prohibiting the endogenous peroxidase, and then washed with phosphate-buffered saline. Sections were incubated for 1 h in the primary antibody (dilution range 1: 100–300). For negative controls, sections were run as routine with skipping the adding of primary antibody. The binding of the primary antibody was disclosed using avidin–biotin–peroxidase detection kit (Dako, USA) as described by the manufacturer's instructions. DAB Chromogenic (1–2 min) was used and slides were counterstained by Harris's hematoxylin, then dehydrated and coverslipped.

Morphological and morphometric analysis

- H and E-stained sections were examined regarding the presence of:
  a. Abnormal proliferation of lymphoid follicles (follicular hyperplasia)
  b. Focal aggregates of lymphoid tissue (reactive lymphoid hyperplasia)
  c. Small or large intraepithelial groups of tightly aggregated lymphocytes located within a vacuole that leading to a defect in the surface epithelium (Urgas's abscess).
- In the other stains, the following parameters were measured using the imaging software analyzer (Image-Pro Plus 2D),

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1. The mean color area percentage of collagen fibers using Masson's trichrome stain
2. The mean count of CD3 positive cells
3. The mean count of CD20 positive cells
4. The mean epithelial thickness of the tonsillar crypt.

All the measurements were taken at five nonoverlapping high-power fields of magnification (×400/section) in three random sections/block.

Statistical analysis

The data collected were processed using one-way analysis of variance of SPSS version 15 (SPSS Inc., Chicago, IL, USA) and *post hoc* significant difference. Quantitative data were expressed as means ± standard deviation. The significance of the data was determined by the *P* value. $P < 0.05$ was considered statistically significant.

Results

Clinical results

There was no statistically significant difference in the mean intensity of CT symptoms severity according to visual analog scale (VAS) before tonsillectomy between Group A and Group B [Table 1]. At the same time, most of patients were clinically in Grade 1+ and 2+ in both groups without any statistically significant difference [Table 2].

Histopathological results

H&E-stained sections of tonsils in Group A (ASOT seropositive) showed marked histopathologic changes in the form of increased thickness (hyperplasia) of the stratified squamous nonkeratinized epithelium that was resting on its basement membrane, in addition to severe lymphocytic infiltration in epithelial and subepithelial areas. The mean epithelial thickness was 174.7, which was significantly higher than that of Group B ($P < 0.0001$) [Figure 1a and Table 3]. Furthermore, marked intraepithelial aggregates of lymphocytes within a vacuole (Urgas's abscess) [Figure 1a], and subepithelium lymphocytic infiltration and congested blood vessels were also seen. Some epithelial cells showed vacuolated cytoplasm and pyknotic nuclei [Figure 1b]. Tonsillar crypts lined with thick stratified squamous nonkeratinized epithelium and infiltrated with lymphocytes were detected. Many enlarged (hyperplastic) lymphoid nodules with prominent reactive pale germinal center and inflammatory infiltrate rich in lymphocytes and hyalinized stroma (enriched in collagen fibers arranged in fascicles) were seen [Figure 1c and d].

H and E-stained sections of tonsils in Group B (ASOT seronegative) showed nonkeratinized stratified squamous epithelium resting on a basement membrane. The mean epithelial thickness was 42.46 [Figure 2a and Table 3]. Lymphatic nodules showed compact aggregates of lymphocytes arranged in the form of dark peripheral ring and pale germinal center. Diffuse lymphocytes were detected between lymphatic nodules [Figure 2b]. Tonsillar crypts lined by thin stratified squamous nonkeratinized epithelium were seen [Figure 2c].

Group A tonsillar sections stained with Masson's Trichrome revealed marked increase in the green collagen fibers, between the lymphoid nodules and around blood vessels. The mean optical density of collagen fibers (632.73) was statistically significant when compared with Group B ($P < 0.0001$) [Figure 3a]. While tonsillar sections in Group B revealed fine collagen fibers in between lymphoid nodules and around blood vessels. The mean optical density of collagen fibers was 206.03 [Figure 3b and Table 4].

Table 1: Mean degree of different visual analog scale symptoms severity in both groups before tonsillectomy

Symptoms	Mean±SD		*t*-test	*P*
	Group A (*n*=15)	Group B (*n*=15)		
Dysphagia	7.6±0.9	8.1±1.4	0.55	0.754
Fever	8.4±0.4	7.9±1.7	0.78	0.439
Arthralgia	7.9±1.2	8.1±0.9	0.18	0.581
Body ache	6.7±0.7	6.2±1.1	0.35	0.709
Recurrent tonsillitis	7.6±1.8	7.2±2.1	0.76	0.764
Obstructive sleep apnea	7.1±1.8	7.6±1.4	0.43	0.617

Insignificant *P*>0.05. SD: Standard deviation

Table 2: Mean degree of tonsillar grade classification in both groups

Tonsillar grade classification	Mean		*t*-test	*P*
	Group A (*n*=15)	Group B (*n*=15)		
Grade 1+	8	7	0.9	0.943
Grade 2+	10	10	0.7	0.759
Grade 3+	1	2	0.9	0.843
Grade 4+	1	1	0.6	0.988

Insignificant *P*>0.05

Table 3: Mean epithelial thickness in both groups

Study groups	Mean±SD	*t*-test	*P*
Group A (seropositive ASLO titer) (*n*=15)	174.7±42.78	92	<0.0001
Group B (seronegative ASLO titer) (*n*=15)	42.46±3.48		

SD: Standard deviation

Table 4: Mean optical density of collagen fibers in both groups

Study groups	Mean±SD	*t*-test	*P*
Group A (seropositive ASLO titer) (*n*=15)	632.73±200.4	76	<0.0001
Group B (seronegative ASLO titer) (*n*=15)	206.03±80.54		

SD: Standard deviation

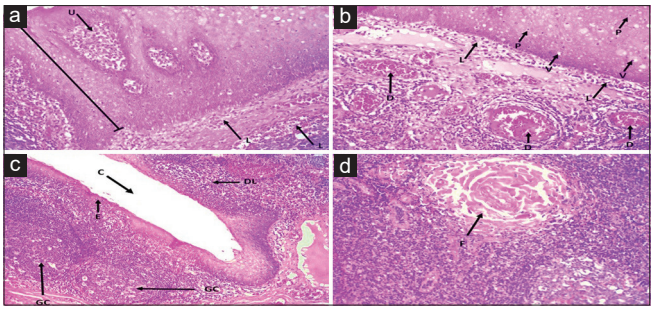

Figure 1: Photomicrograph of a tonsil of seropositive group, showing (a) hyperplastic stratified squamous non-keratinized epithelium (Line), Urgas's abcess (U), lymphocytic infiltration in both epithelial & subepithelial areas are shown (L).(H&E X200). (b) Massive subepithelial inflammatory infiltrates (L), dilated congested blood vessels (D) and some epithelial cells with vacuolated cytoplasm (V) and pyknotic nuclei (P) are shown.(H&E X200). (c) Tonsillar crypts (C) lined with thick epithelium (E) infiltrated with lymphocytes, diffuse Lymphocytes (DL) and lymphoid nodules with prominent germinal centers are shown (GC).(H&E X100). (d) Inflammatory infiltrate rich in lymphocytes and hyalinized stroma (enriched in collagen fibers arranged in fascicles) also shown (F). (H&E X400)

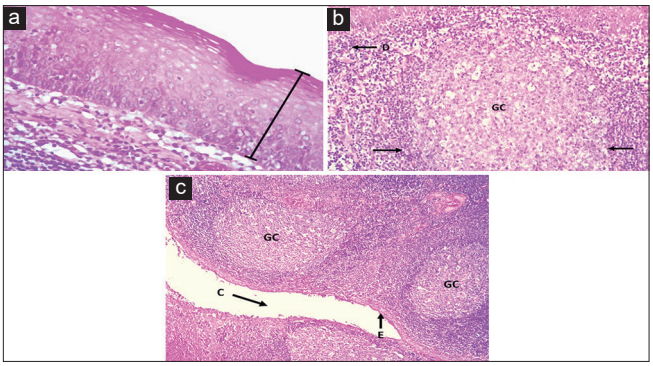

Figure 2: Photomicrograph of a tonsil of seronegative group, showing (a) non-keriatinized stratified squamous epithelium with a normal thickness (Line) resting on a basement membrane. (H&E X400). (b) Lymphoid nodule containing a pale germinal center (GC) and a dark peripheral ring of lymphatic cells (Arrows) and diffuse lymphocytes are also shown (D) (H&E X200). (c) Tonsillar Cyrpts (C) lined with thin epithelium (E) and lymphoid nodules with a pale germinal centers (GC) and dark peripheries are shown. (H&E X100)

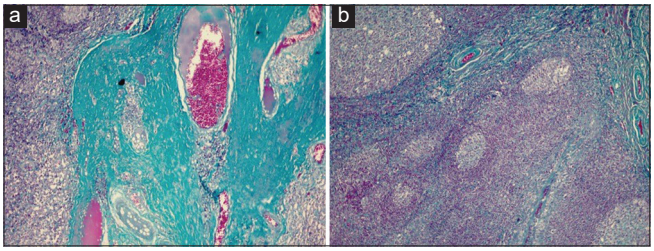

Figure 3: Photomicrograph of a tonsil of: (a) Seropositive group, showing marked increase in the amount of collagen fibers around blood capillaries. (b) Seronegative group, showing fine collagen fibers in between lymphatic nodules and around blood capillaries. (Masson's Trichrome X100)

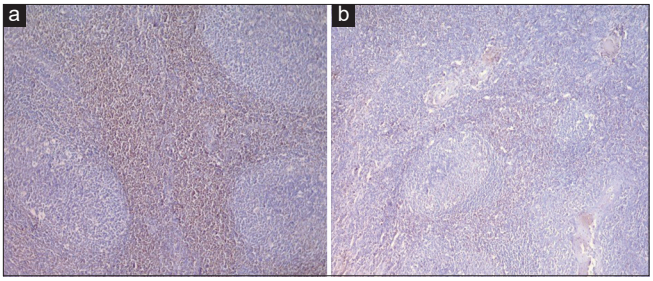

Figure 4: Photomicrograph of a tonsil of: (a) Seropositive group, showing moderate increase in CD3 expression between lymphatic nodules. (b) Seronegative group, showing mild CD3 expression between lymphatic nodules. (DAB Harris's, hematoxylin counterstaining X100)

Using immunohistochemical technique, tonsillar expression of CD3 (T-lymphocytes) in Group A appeared significant increase in the brown color reaction in between the lymphoid nodules when compared to Group B ($P < 0.0001$). The mean optical density of CD3 was 522.74 [Figure 4a]. While Group B showed mild brown color reaction in between lymphoid nodules. The mean optical density of CD3 was 272.82 [Figure 4b and Table 5].

Using immunohistochemical technique, tonsillar expression of CD20 (B-lymphocytes) in Group A indicated significant rise in the brown color reaction within the lymphoid nodules when compared to Group B ($P < 0.0001$). The mean optical density of CD20 was 256.53 [Figure 5a]. Group B showed faint brown color reaction within the lymphoid nodules. The mean optical density was 43.53 [Figure 5b and Table 6].

Discussion

CT is one of the most frequent chronic inflammation of the palatine tonsils affecting children that requiring surgical removal. The current study was designed to assess the expression of T- and B-lymphocytes (tonsillar lymphoid nodules) in relation to ASOT seropositive or seronegative in thirty children (6–10 years) diagnosed as CT and were subjected to conventional dissection tonsillectomy in ENT Department, Faculty of Medicine, Jazan University, Kingdom of Saudi Arabia, from October 2017 to April 2018.

Antistreptolysin antibodies are produced about 7:30 days following a *GABHS* infection. The ASOT antibody titer peaks at about 3–5 weeks after the illness and then tapers off but may remain detectable for several months after the streptococcal infection has resolved.[10] In the current study, the tonsillar histopathological changes in Group A were in the form of marked lymphoid hyperplasia and lymphocytic infiltration in epithelial and subepithelial areas and Urgas's abscess in the surface epithelium. Tonsillar crypts lined by thick stratified squamous nonkeratinized epithelium and increased amount of collagen fibers between the lymphoid nodules and around blood vessels were observed. While; Group B showed significant decrease in epithelial thickness in surface epithelium and tonsillar crypts, diffuse lymphocytes were found in between lymphoid nodules, and significant decreased amount of collagen fibers between the lymphoid nodules and around blood vessels. These results were in accordance with

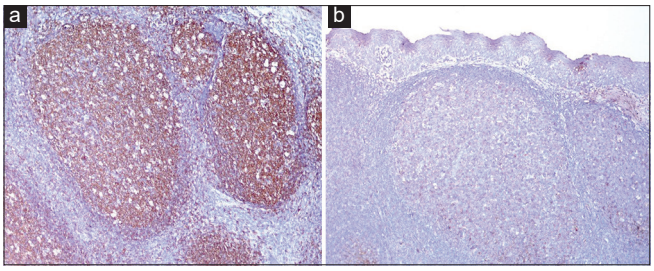

Figure 5: Photomicrograph of a tonsil of: (a) Seropositive group, showing marked increase in CD20 expression in lymphatic nodules. (b) Seronegative group, showing faint CD20 expression in lymphatic nodules. (DAB Harris's, hematoxylin counterstaining X100)

Table 5: Mean optical density of CD3 (T-lymphocytes) in both groups

Study groups	Mean±SD	*t*-test	*P*
Group A (seropositive ASLO titer) (*n*=15)	522.74±151.2	48	<0.0001
Group B (seronegative ASLO titer) (*n*=15)	272.82±13.02		

SD: Standard deviation

Table 6: Mean optical density of CD20 (B-lymphocytes) in both groups

Study groups	Mean±SD	*t*-test	*P*
Group A (seropositive ASLO titer) (*n*=15)	256.53±51.55	15	<0.0001
Group B (seronegative ASLO titer) (*n*=15)	43.53±1.33		

SD: Standard deviation

previous studies that evinced the same histopathological changes without focusing on serological results of these patients.[11-13]

In the present study, immunohistochemical results showed marked expression of CD3 in between the tonsillar lymphoid nodules in Group (A), compared to mild expression in Group (B). However, CD20 expression showed marked increase within the tonsillar lymphoid nodules in Group (A) compared to negative expression in Group (B). Similar results were demonstrated by Mogoanta *et al.,* who stated that the T-lymphocytes localized preponderantly at the perifollicular region and surface epithelium. On the other hand, they found that B-lymphocytes were present within the lymphoid nodules.[4]

The histopathological and immunohistochemical results of the current study could be explained by Miroljub and Elvir's study, which showed that in CT, T-lymphocytes produced proinflammatory cytokines. T-lymphocytes started with the secretion of IFN-γ and TNF-α cytokines from Th1 cells, followed by secretion of the anti-inflammatory cytokines (IL-4 and IL-6) from Th2 cells as a result of overproduction caused by continual stimulation by GABHS. Th1 cells stimulate cell mediated immune response, and stimulate B cells to produce antibodies. While, Th2 cells stimulate humoral immune response, and promotes B cell proliferation.[14]

In the current study, the serological results were in accordance with the histopathological and immunohistochemical results, in which positive ASOT in Group A indicated recent streptococcal infection, which, in turn, reflected marked increase in expression of CD 20 and CD3 in the tonsillar tissue. However, negative ASOT in Group B indicated null streptococcal infection and consequences negative expression of CD 20 and CD3 in the tonsillar tissue.

Conclusion

Seropositive ASOT CT, in addition to histopathological changes, is associated with significant increase in both B-lymphocytes (CD20 expression) and T-lymphocytes (CD3 expression) markers. Further work should be done to explain the exact mechanisms that lead to increase of CD3 and CD20 expression in seropositive ASOT CT.

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Nil.

Conflicts of interest

There are no conflicts of interest

References

1. Randel A. AAO-HNS guidelines for tonsillectomy in children and adolescents. Am Fam Phys 2011;84:566-73.
2. Yamanaka N. Moving towards a new era in the research of tonsils and mucosal barriers. Adv Otorhinolaryngol 2011;72:6-19.
3. Geibler K, Markwart R, Requardt RP, Weigel C, Schubert K, Scherag A, *et al*. Functional characterization of T-cells from palatine tonsils in patients with chronic tonsillitis. PLoS One 2017;12:e0183214.
4. Mogoanta CA, Ionita E, Pirici D, Mitroi M, Anghelina FL, Ciolofan S, *et al*. Chronic tonsillitis: histological and immunohistochemical aspects. Rom J Morphol Embryol 2008;49:381-6.
5. Machado CS, Ortiz K, Ade LM, Martins RS, Machado NC. Antistreptolysin O titer profile in acute rheumatic fever diagnosis. J Pediatr (Rio J) 2001;77:105-11.
6. Hembrom R, Roychaudhuri BK, Saha AK, Roychowdhury A, Ghosh S, Gon S, *et al*. Evaluation of the validity of high serum antistreptolysin o titre only, as an indication for tonsillectomy. Indian J Otolaryngol Head Neck Surg 2014;66:232-6.
7. Fry L, Baker BS. Triggering psoriasis: the role of infections and medications. Clinics in Dermatology. 2007;25:606-15.
8. Dell'Aringa AR, Juares AJ, Melo C, Nardi JC, Kobari K, Perches R. Histological analysis of tonsillectomy and adenoidectomy specimens-January 2001 to May 2003. Braz J Otorhinolaryngol 2005;71:18-22.
9. Danchin MH, Carlin JB, Devenish W, Nolan TM, Carapetis JR. New normal ranges of antistreptolysin O and antideoxyribonuclease B titres for Australian children. J Paediatr Child Health 2005;41:583-6.
10. Kotby AA, Habeeb NM, Elarab SE. Antistreptolysin O titer in health and disease: Levels and significance. Pediatr Rep 2012;4:e8.
11. Reis LG, Almeida EC, da Silva JC, Pereira Gde A, Vde BF, Etchebehere RM. Tonsillar hyperplasia and recurrent tonsillitis: clinical-histological correlation. Braz J Otorhinolaryngol 2013;79:603-8.
12. Uğraş S, Kutluhan A. Chronic tonsillitis can be diagnosed with histopathologic findings. Eur J Gen Med 2008;5:95-103.
13. Alatas N, Baba F. Proliferating active cells, lymphocyte subsets, and dendritic cells in recurrent tonsillitis: their effect on hypertrophy, Arch Otolaryngol Head Neck Surg 2008;134:477-83.
14. Miroljub MT, Elvir ZZ. Immunoregulatory cytokines and chronic tonsillitis. Bosn J Basic Med Sci 2013;13:230-6.